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STUDIES ON WEAK ELECTROMAGNETIC FIELDS EFFECTS IN CHICK
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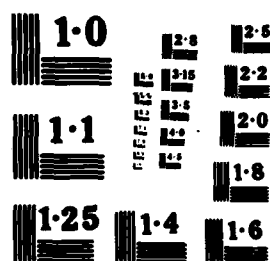
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) This year, our research was directed to test some experimental conditions of the Henhouse project and to enforce our previous study on VLF electromagnetic fields effects on chick embryos. Henhouse Project: - We studied the response of White Leghorn Hisex embryos to field exposures effective on the Shaver breed, as previously reported. (1) A 48 hours exposure, in vivo, to a pulsed horizontal field of 100 Hz frequency, 1.0 mT intensity, 500 μ sec pulse duration and 2 μ sec rise time induced a significant increase of developmental abnormalities in Hisex embryos. (2) A five hours exposure of stage 7 Hisex embryos changed the Mitotic Index of their neural tissue. So, the early development of Hisex embryos, like Shaver embryos, can be modified by VLF pulsed electromagnetic fields. - In the protocol of the Henhouse project, it was suggested a temperature of 38°C for eggs in-			
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incubation. Studying the development of chick embryos in relation to the temperature, in the range of 37.4-40°C, we confirmed that a 48 hours incubation at 38°C (with 55% humidity) does not induce abnormalities and allows a convenient developmental growth rate of the chick embryos.

Electromagnetic Fields effects in relation to the embryos orientation:

Our preliminary results on the induction of abnormalities in field exposed embryos in relation to their orientation were confirmed. In a East-West oriented horizontal pulsed field, the organisms oriented to Southwest and Southeast showed a significant increase of developmental abnormalities. No effect was appreciable among the embryos Southward oriented.

Remark:

For a period of time, we could not duplicate our reported results on electromagnetic field effects on embryonic development. But now, the experiments give reproducible results as previously observed and reported. We are trying to find the causes of this temporary lack of effect.

ANNUAL SCIENTIFIC REPORT

STUDIES ON WEAK ELECTROMAGNETIC FIELDS EFFECTS
IN CHICK EMBRYOS

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INTRODUCTION

The subject of our research is the effects of exposure to very low frequency and very low intensity electromagnetic fields (EMF_s) on the embryonic development.

The complexity of this matter lies on the difficulty to reproduce our reported results, probably due to the multiple parameters which influence the incidence of the fields on the organisms. Some teams have partially succeeded in this reproduction (J. Juutilainen, et. al 1986), although they used higher intensities than those found effective in our studies; other teams failed in their attempts (S. Maffeo, et. al 1984, R. Tell, personal communication).

In order to solve the problems of reproductiveness, we have approached our work on some parameters, such as the breed of the hens, the orientation of the embryos inside the field, the conditions of incubation and the conditions of exposure to the EMF.

EXPERIMENTAL SYSTEMS AND MATERIALS

Our biological system consists of early White Leghorn chicken embryos. In previous studies, we used the Shaver breed. Now, we are repeating some of the experiments on Hisex embryos, this breed being the one selected for experimentation in the Henhouse project.

We study in vivo, the development of chick embryos incubating the eggs for two days after laying at 38°C, with 55% humidity, in a Memmert incubator.

We used two incubators, one of them for the field exposed eggs, the other for the controls. In the first one called "Experimental incubator", are located cylindric coils or Helmholtz coils inside which are put the experimental eggs. When the coils are stimulated, we detect outside them, in different zones of the incubator, field intensities reaching 0.4 to 2.2% values of the field induced in the coils. In some experimental conditions, this contamination outside the coils has a value shown effective on embryonic development and in other cases values which effectivity is not known.

No difference was seen between the samples of embryos incubated inside coils located in the experimental incubator, and the embryos incubated outside coils in the control incubator. So, we adopt in our experimental protocol the use of two incubators, the experimental embryos being inside coils and the control embryos out-

side coils.

The different conditions that can determine the sensitivity of the embryos to the artificial change of their environment, for example, their orientation in the field, the duration of the exposure, the period of the developmental stages, are studied through a statistical methodology comparing the proportions of abnormal embryos in experimental and control samples. At the present time, in such studies only the morphology of the embryos is analyzed. In other subjects of research, such as the incidence of pulsed electromagnetic field on cellular division, the study implies histological preparations which are analyzed.

HENHOUSE PROJECT

For technical reasons, the project could not be started yet.

Furthermore, we began to study with our material (Memmert incubators, "home made" Helmholtz coils, Grass S.D. 9 stimulator...) some field exposure conditions which could be determinant.

In the collaborative Henhouse project, the embryos will be of the White Leghorn Hisex breed, exposed 48 hours to a field of 100 Hz frequency, 1.0 μ T intensity, 500 μ sec pulse duration and 2 μ sec pulse rise time. The incubation will be done at 38°C with 55% humidity.

As we previously studied and described the effects of this field with a 0.4 μ T intensity on White Leghorn embryos of the Shaver breed, we worked this year (1) to repeat our experiments comparing the effects of this field at 0.4 μ T and 1.0 μ T intensity on Hisex embryos, (2) to determine a temperature limit of egg incubation, preventing an increase of abnormalities in the experimental as well as in the control embryos, and to control the possible incidence of this factor on our previous results.

- Effects of the field at 0.4 and 1.0 μ T intensities on White Leghorn Hisex embryos: The experiments have shown conflicting results at 0.4 μ T and at 1.0 μ T. We had problems to reproduce our reported results. Previously, in each experiment, the percentage of abnormal embryos was higher in the sample exposed to the field at 0.4 μ T intensity than in the control one. It was not the case this year although the experimental conditions were exactly the same as those previously used and described. Some experiments showed a teratogenic effect of the fields, some others were not effective and others showed a favorable effect. The breed was not responsible: with Shaver as with Hisex embryos we observed changes of the effect from one experiment to other. Evidently, these changes can be due to chance in experiments with a field without effect. But the difference of these results with those we previously obtained under the same conditions, obliged us to research the factor(s) responsible for these changes. The study is in progress.

At the present time, the experiments show a regular, repetitive teratogenic effect of the fields on chick embryos development, and we are studying the incidence of the treatment in relation to the topological localization of the eggs inside the Helmholtz coil. For this purpose, a physical analysis has been made on the characteristics of the field inside the coil. Preliminary results show that the embryos exposed to the field at a $1.0 \mu T$ intensity, in the center of the coil, are strongly disturbed by the exposure. Up to date the experimental sample (35 embryos) showed a 5 times increase of abnormalities than in the control one (51 embryos) with $p = 0.0016$. The main abnormalities induced by the field are malformed Cephalic Nervous System ($p = 0.009$) and abnormal truncal flexure ($p = 0.007$).

- Effects of temperature on chicken embryos development: As explained in our progress report, we performed these experiments as it was suggested in a meeting in Arlington (FDA) in November 1984.

The embryos were incubated in two different ovens. One of them (the control incubator) was always kept at $37.6 \pm 0.2^\circ C$. In the other (the experimental incubator), we tested temperatures between $37.4 \pm 0.2^\circ C$ and $41.0 \pm 0.2^\circ C$.

1.- In a first series of experiments, the embryos were incubated during 48 hours at an experimental temperature between 37.4 and $39.9^\circ C$ and compared at the end of the two days with their controls, simultaneously incubated, in all the cases, at $37.6 \pm 0.2^\circ C$. No change was seen in the proportion of abnormals among the embryos incubated at temperatures between 37.4 and $38.5^\circ C$ (182 experimental embryos, 32 abnormal, were compared to 132 controls being 27 abnormal; $p = 0.528$). At temperatures between 38.7 and $39.9^\circ C$, the proportion of abnormals was increased (151 experimental embryos, 31 abnormal, compared to 109 controls, 12 being abnormal; $p = 0.041$).

The mean developmental stage of the experimental samples incubated at temperatures between $38.5^\circ C$ and $39.9^\circ C$ was always higher than the mean stage of the control groups. A two stage advancement was observed when the embryos were incubated between 39.5 and $39.9^\circ C$.

According to these results $38.5^\circ C$ could be considered as a limit temperature for a two days incubation of fertilized chicken eggs in these experimental conditions. In our previous and present studies of EMFs effects on chick embryos development, the incubation of the fertilized eggs has been done at $38^\circ C$, which ensures a correct growth of the organisms and does not induce abnormalities.

2.- In a second series of experiments, we observed that the hyperthermia at $39.7 \pm 0.2^\circ C$ applied only during the second day of the incubation period, induced a significant increase of abnormalities in the embryonic development (67 experimen-

tal embryos, 21 being abnormal were compared to 69 controls, 8 of them abnormal; $p = 0.006$). This effect was not observed when the hyperthermia ($39.7 \pm 0.2^\circ\text{C}$) was applied only during the first day (56 experimental embryos, 5 abnormal and 69 controls, being 8 abnormal; $p = 0.771$). In both cases of hyperthermic treatment, the development of the embryos was advanced in comparison with their controls incubated two days at $37.6 \pm 0.2^\circ\text{C}$. The abnormalities induced by the hyperthermia at $39.7 \pm 0.2^\circ\text{C}$ were opened and malformed Central Nervous System, mainly in the truncal part.

When a higher temperature ($41.0 \pm 0.2^\circ\text{C}$) was applied the second day of incubation, the effect was statistically more drastic on the samples and the abnormalities more severe (57 experimental embryos showing 28 abnormal, compared to 54 controls, 4 of them abnormal; $p < 0.0005$).

THE ORIENTATION OF THE ORGANISM, POSSIBLE FACTOR DETERMINANT FOR THE VLF ELECTROMAGNETIC FIELDS INCIDENCE OF EMBRYONIC DEVELOPMENT.

In our experimental system, a pulsed and horizontal field oriented East-West is applied to fertilized eggs. In the eggs of the White Leghorn Shaver breed, the embryos are found mainly oriented in three directions which correspond to the geomagnetic Southwest (SW), South (S) and Southeast (SE). Our previous results on EMFs effects on chick embryos in vivo, suggested that, when a field causes teratogenic effects on a population, the proportion of anomalies in the development increased among the organisms oriented to SW and SE. Not any teratogenic effect was appreciable on the embryos oriented Southward, orientation perpendicular to the artificial pulsed field.

To confirm this result, we decided to study the embryos oriented to the North, the other direction perpendicular to the field. So, fertilized eggs of the White Leghorn Shaver breed were exposed to the same field used at the beginning of this work and in the same conditions: the eggs were located in five cylindric coils, their narrow end Westward, the field had a 100 Hz frequency, $1.0 \mu\text{T}$ intensity, 500 μsec pulse duration and an approximately 100 μsec pulse rise time. We recall that the orientation of the embryos in the eggs was always determined before the morphological analysis of the embryo, therefore before the determination of their normality or abnormality and of their developmental stage. The results were the following:

- (1). The field exposure had a slight but significant teratogenic effect on the embryonic development. 123 embryos were exposed 48 hours to this EMF and 54 of them developed abnormalities (43.9%) while among 386 controls, 113 were found abnormal (29.2%; $p = 0.003$).
- (2). In these experiments we could determine the orientation of 103 field exposed embryos (63 normals and 40 abnormal) and 321 controls (235 normals and 86 abnormal).

mals). Actually some embryos were not taken in account for the orientation study for different reasons, for example, accidental moving of the egg when it was taken out of the incubator, a shell relatively stuck to the yolk provoking movements of the yolk during the window-opening of the shell, under developed embryos which orientation could be determined but with a large margin of error.

In the experimental sample, only one embryo was found oriented Northward representing 0.9% of the population. It was normal. In the control group, 2 embryos were in this orientation, representing 0.6% of the population. One of them was normal, the other abnormal. The study on the Northward oriented organisms could not be made in such in vivo experimental conditions.

(3). In the experimental population the percentage of abnormal embryos SW and SE oriented was 2 times increased with respect to the controls in each of these orientations ($p = 0.005$ in SW; $p = 0.035$ in SE) and approximately two times decreased with respect to the controls, although not significantly, in the South direction. The percentage of the total population in each of these orientations was not changed. These results confirm our preliminary findings and suggest a relationship between the orientation of the organisms and the teratogenic incidence of the pulsed EMF on their development.

EXPOSURE TO ELF PULSED ELECTROMAGNETIC FIELD AND MULTIPLICATION RATIO OF THE EMBRYONIC NEURAL TISSUE.

The first study was performed on White Leghorn embryos of the Shaver breed. As in the Henhouse project, it was decided to use Hisex embryos, we tested on stage 7 Hisex embryos, the response of the neural tissue to a 5 hours field exposure.

We found equivalent values of Mitotic Index (MI) in the cephalic zone of the neural tissue in Hisex and Shaver control embryos at the same developmental stage. In the truncal zone of the tissue more variability of the measures was found. Significant changes of the MI in field exposed embryos were confirmed using the Hisex breed. We also confirmed the special sensitivity of the Rhombencephalic zone, where the value of the MI was increased. (The statistic tests used were the comparison of percentages and the Wilcoxon test). This result could be related with an histological abnormality only found in these field exposed embryos: An abnormally extensive dorsal growth of the neural tissue, provoking a partial occlusion of the lumen, which occurred in all cases in the Rhombencephalon.

At the end of the 5 hours exposure or after a 5 hours post-incubation period without field, no change was appreciated in the gross morphology of the embryos. But significant changes were seen with prolonged field exposure (study in progress).

So, this year our research was directed to resolve technical questions of the Hen-house project protocol and to enforce the study on the field effects in relation to the embryos orientation. The temporary lack of effect of the EMF exposure delayed the work and showed the complexity of this subject of research.



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